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Research Article

Two new *Neohelicomyces* species (Tubeufiaceae, Tubeufiales) associated with *Coffea arabica* L. in Yunnan Province, China

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Abstract

Neohelicomyces species comprise a group of helicosporous hyphomycetes, with over 90% of the currently accepted taxa reported from China, occurring in both freshwater and terrestrial habitats. Although the genus has been increasingly documented in recent years, its presence in crop-related ecosystems remains poorly understood, as most species have been reported from unknown hosts. In this study, a survey of fungi associated with *Coffea arabica* in Yunnan Province, China, was conducted, and fruiting bodies of helicosporous hyphomycetes were found on dead branches of coffee plants. Based on the morphological characteristics of conidiophores, conidiogenous cells, and conidia, in combination with multigene phylogenetic analyses (ITS, LSU, *rpb2*, and *tef*1-α), two novel species of *Neohelicomyces* (*N. coffeæ* and *N. puerensis*) were identified. Morphologically, *N. coffeæ* differs from closely related species *N. edgeworthiæ* by having shorter conidiophores, longer conidiogenous cells, and smaller, multi-septate conidia (vs. aseptate in *N. edgeworthiæ*), while *N. puerensis* differs from its close relative *N. dehongensis* by having narrower and distinctly multi-septate conidia, more tightly coiled conidial filaments, and unbranched conidiophores. These results expand the known diversity of *Neohelicomyces* and contribute to a better understanding of fungal assemblages associated with coffee plants in subtropical China. In addition, detailed descriptions, illustrations, and phylogenetic trees are provided.

Key words: Coffee-associated microfungi, morphology, multigene phylogeny, new taxa, taxonomy

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Introduction

Yunnan Province in southwestern China is a global biodiversity hotspot, supporting an exceptionally high diversity of plants and fungi (Hyde et al. 2020; Qian et al. 2020; Shen et al. 2022; Zhu and Tan 2022; Hongsanan et al. 2023). Among its major crops, coffee (*Coffea arabica* L.) is particularly important, with Yunnan accounting for nearly all of China's coffee production (Wang et al. 2025). However, coffee plants are highly susceptible to colonization by diverse fungi, which can function as pathogens, endophytes, or saprobes, thereby influencing plant health, yield, and ecological dynamics (McCook and Vandermeer 2015; Hyde et al. 2020; Lu et al. 2022a, 2025a, 2025b). Recent surveys have revealed rich fungal assemblages associated with coffee in Yunnan, highlighting both the ecological uniqueness of the region and the economic significance of the crop (Lu et al. 2025a, 2025b; Wang et al. 2025). These factors underscore the need for continuous investigations of coffee-associated fungi in Yunnan to better understand fungal diversity and its implications for sustainable coffee production (Lu et al. 2025a, 2025b).

Tubeufiales was established by Boonmee et al. (2014) to accommodate Tubeufiaceae, which molecular phylogenetic analyses demonstrated to represent an independent lineage within Dothideomycetes, distinct from previously established orders. The circumscription of the order was supported by both morphological features and multigene phylogenies incorporating LSU, ITS, and *tef*1-α sequences (Boonmee et al. 2014). At present, Tubeufiales comprises three families: Tubeufiaceae, the type and largest family originally delineated by Barr (1979), along with Bezerromycetaceae and Wiesneriomycetaceae, which were subsequently established based on divergence-time estimates and molecular phylogenetic evidence by Liu et al. (2017). Members of the order are cosmopolitan in distribution, occurring across tropical, subtropical, and temperate regions, where they function predominantly as saprobes on decaying woody substrates in both terrestrial and freshwater habitats, particularly in aquatic environments such as streams, lakes, and swamps (Boonmee et al. 2014; Liu et al. 2017; Lu et al. 2018b; Ma et al. 2024a, 2024b; Sun et al. 2025).

The family Tubeufiaceae is a significant group within the fungal kingdom, which was established by Barr (1979) based on the generic type *Tubeufia*. This family was created to accommodate a group of fungi that exhibit both sexual and asexual morphs, with the asexual forms predominantly being helicosporous hyphomycetes (Lu et al. 2018b; Dong et al. 2020), while the sexual morph is characterized by superficial ascomata, pseudoparaphysate hamathecium, and multiseptate, hyaline to pale brown cylindrical ascospores (Barr 1980; Kodsueb et al. 2006; Boonmee et al. 2011, 2014; Brahmanage et al. 2017; Lu et al. 2018b; Ma et al. 2024a, 2024b; Peng et al. 2025; Sun et al. 2025). Members of the Tubeufiaceae are predominantly saprobic, decomposing wood and other organic matter in both terrestrial and freshwater habitats (Lu et al. 2018b; Ma et al. 2024a, 2024b; Ma et al. 2025). They are widely distributed across tropical and subtropical zones (Cai et al. 2002; Liu et al. 2018; Lu et al. 2018a, 2018b). Tubeufiaceae has been the subject of numerous taxonomic revisions and expansions in recent years. Lu et al. (2018b) provided an updated phylogenetic tree for Tubeufiales, which included 13 genera, significantly expanding the circumscription of the type family Tubeufiaceae. Subsequently, the family has been continuously refined through subsequent research, with the introduction of new genera, such as *Neo-*

manoharachariella (Li et al. 2022) and *Pseudotubeufia* (Ma et al. 2023). As of now, Tubeufiaceae comprises 47 genera, including well-known genera such as *Tubeufia*, *Helicoma*, *Helicosporium*, and *Neohelicomyces* (Hyde et al. 2024).

Neohelicomyces was established by Luo et al. (2017) based on morphological characteristics and phylogenetic analyses of combined ITS, LSU, and *tef*-1α sequence data, with *N. aquaticus* as the type species, and the genus comprises helicosporous hyphomycetes defined by coiled and helical conidia (Luo et al. 2017; Lu et al. 2018b, 2022b; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024a, 2024b). Morphologically, species of *Neohelicomyces* are characterized by macronematous, mononematous, erect, septate conidiophores, usually fertile in the lower half and sterile towards the apex, monoblastic, holoblastic, and integrated conidiogenous cells bearing lateral minute denticles that produce a single conidium each, with helicoid, multiseptated conidia, smooth-walled, pale brown, guttulate, with filaments that are tightly to loosely coiled (2–2.5 times), rounded at both ends (Luo et al. 2017; Lu et al. 2018b, 2022b; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024a, 2024b). Morphologically, *Neohelicomyces* is similar to *Helicomyces*, *Helicosporium*, and *Neohelicosporium*, but it differs from *Helicomyces* by possessing elongate, erect, and conspicuous conidiophores, and can be distinguished from *Helicosporium* by differences in conidial coiling ratio and size, and differs from *Neohelicosporium* by macronematous, mononematous, erect, septate and often conspicuous conidiophores arising directly on the substrate, with conidiogenous cells that are integrated and denticulate, and helicoid conidia that are relatively large and coiled (Tsui et al. 2006; Luo et al. 2017; Lu et al. 2018a).

In this study, fruiting bodies of two helicosporous taxa were collected and isolated from dead coffee branches in Pu'er City, Yunnan Province, China. Based on morphological characteristics, illustrations, and multi-gene phylogenetic analyses, the two taxa were identified as novel species within *Neohelicomyces*, namely *N. coffeæ* and *N. puerensis*.

Materials and methods

Specimen collection, examination, and isolation

Pu'er City is one of the major coffee-growing regions in China, characterized by a warm and humid subtropical monsoon climate that provides favorable conditions for both coffee cultivation and fungal diversity (Zhang et al. 2020). In this study, sampling targeted decaying branches of *C. arabica* bearing white fungal fruiting bodies in Pu'er City, Yunnan Province, China. Samples were placed in self-sealing plastic bags, with detailed collection data recorded in the field (Rathnayaka et al. 2025) and subsequently transported to the mycology laboratory at Qujing Normal University, China. The external features of fruiting bodies on specimen surfaces were first examined using a stereomicroscope (OLYMPUS SZ61, Japan). Micromorphological features were observed using a compound microscope (OLYMPUS BX53, Olympus, Tokyo, Japan), and images were captured with an OLYMPUS DP74-CU digital camera. Measurements were performed using Tarosoft® Image Framework v. 0.9.7 software, and final photographic plates were prepared using Adobe Photoshop CC 2019 v. 23.1.0 (Adobe Systems, USA).

Pure cultures were isolated through single-spore isolation, following the procedures described by Senanayake et al. (2020). Germinated conidia were transferred to fresh potato dextrose agar (PDA) plates and incubated at 28 °C for 2–6 weeks, after which colony morphology was examined. Dried herbarium specimens were preserved in the Herbarium of Guizhou Medical University (GMB-W, Guiyang, China), while living cultures were deposited in the Guizhou Medical University Culture Collection (GMBCC, Guiyang). Index Fungorum (IF) numbers were assigned following Index Fungorum (2026).

DNA extraction, PCR amplification, and sequencing

Fresh mycelia were scraped from the surface of colonies (2–6 weeks grown on PDA) with a sterilized toothpick and transferred to a 1.5 ml microcentrifuge tube for DNA extraction. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit-BSC14S1 (BioFlux, China), following the manufacturer's protocol. Primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), EF1-983F/EF1-2218R (Rehner and Samuels 1994), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify ITS, LSU, *tef*1-α, and *rpb*2 sequence fragments, respectively. The PCR reactions were carried out in a total volume of 25 µL, containing 12.5 µL of 2 × Bench Top™ Taq Master Mix, 8.5 µL of ddH_2O, 1 µL of each forward and reverse primer, and 2 µL of DNA template. PCR amplification of the ITS, LSU, and *tef*1-α regions was performed under the following conditions: an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 50 s, and extension at 72 °C for 60 s, with a final extension at 72 °C for 10 min. For *rpb*2, PCR was conducted under similar conditions, except for an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 1 min, 55 °C for 2 min, and 72 °C for 90 s, with a final extension at 72 °C for 10 min. The PCR products were purified and sequenced by Sangon Biotechnology Co., Ltd. (Shanghai, China). All new sequences generated in this study were submitted to GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and are detailed in Table 1.

Phylogenetic analyses

Raw forward and reverse reads generated in this study were first checked for quality and assembled using BioEdit v. 7.0.5.3 (Hall 1999) and SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA) (Swindell and Plasterer 1997). The sequences incorporated in this study were downloaded from GenBank (https://www.ncbi.nlm.nih.gov). Each locus was individually aligned using the MAFFT v.7 online server (https://mafft.cbrc.jp/alignment/server/) (Katoh et al. 2019), and poorly aligned or ambiguous regions were removed with trimAl v. 1.2 (Capella-Gutiérrez et al. 2009), followed by manual adjustments in BioEdit v. 7.0.5.3. The multilocus datasets (ITS+LSU+*rpb*2+*tef*1-α) were concatenated in SequenceMatrix v. 1.7.8 (Vaidya et al. 2011). Maximum likelihood (ML) and Bayesian inference (BI) analyses were carried out on the CIPRES Science Gateway platform (https://www.phylo.org/portal2/home.action) (Dissanayake et al. 2020). The ML tree was inferred using RAxML-HPC v.8 on XSEDE (Stamatakis 2006), applying the GTRGAMMA model with 1,000 bootstrap replicates. BI tree was implemented in MrBayes v. 3.2.7a on XSEDE (Huelsenbeck and Ronquist 2001;

Table 1. Taxa used in this study, along with their corresponding GenBank accession numbers. The newly generated sequences are in bold black. After the strain number, "T" indicates the type strains. "NA" indicates sequence unavailability.

Fungal species	Strain numbers	ITS	LSU	*tef*1-α	*rpb*2
Bezerromyces brasiliensis	CBS 141545	NR_153463	NG_069376	NA	NA
Bezerromyces brasiliensis	URM7411	KX470390	KX518623	KX518631	NA
Muripulchra aquatica	DLUCC 0571	KY320531	KY320548	NA	NA
Muripulchra aquatica	KUMCC 15-0245	KY320533	KY320550	KY320563	MH551057
Muripulchra aquatica	KUMCC 15-0276	KY320534	KY320551	KY320564	MH551058
Muripulchra aquatica	MFLUCC 15-0249^{T}	KY320532	KY320549	NA	NA
Neohelicomyces acropleurogenus	CGMCC 3.25549^{T}	PP626594	PP639450	PP596351	PP596478
Neohelicomyces aquaticus	KUMCC 15-0463	KY320529	KY320546	KY320562	MH551065
Neohelicomyces aquaticus	MFLUCC 16-0993^{T}	KY320528	KY320545	KY320561	MH551066
Neohelicomyces aquisubtropicus	GZCC 23-0080^{T}	PQ098499	PQ098537	PV768327	PV768336
Neohelicomyces aquisubtropicus	GZCC 24-0163	PV730410	PV730414	PV768328	PV768337
Neohelicomyces aseptatus	CGMCC 3.25564^{T}	PP626595	PP639451	PP596352	PP596479
Neohelicomyces coffeae	**GMBCC 2225^{T}**	**PX308843**	**PX308848**	**PX314510**	**PX314514**
Neohelicomyces coffeae	**GMBCC 2226**	**PX308844**	**PX308849**	**PX314511**	**PX314515**
Neohelicomyces dehongensis	MFLUCC 18-1029^{T}	NR_171880	MN913709	MT954393	NA
Neohelicomyces denticulatus	GZCC 19-0444^{T}	OP377832	MW133855	NA	NA
Neohelicomyces denticulatus	UAMH 10535	AY916462	AY856913	NA	NA
Neohelicomyces denticulatus	GZCC 23-0073^{T}	PP626596	PP639452	PP596353	PP596480
Neohelicomyces deschampsiæ	CPC 33686^{T}	MK442602	MK442538	NA	NA
Neohelicomyces edgeworthiæ	CGMCC 3.25565^{T}	PP626597	PP639453	PP596354	PP596481
Neohelicomyces grandisporus	KUMCC 15-0470^{T}	KX454173	KX454174	NA	MH551067
Neohelicomyces guizhouensis	GZCC 23-0725^{T}	PP512969	PP512973	PP526727	PP526733
Neohelicomyces guizhouensis	GZCC 23-0726	PP512970	PP512974	PP526728	PP526734
Neohelicomyces guttulatus	CGMCC 3.25550^{T}	PP626598	PP639454	PP596355	NA
Neohelicomyces guttulatus	GZCC 23-0406	PP626599	PP639455	PP596356	PP596482
Neohelicomyces hainanensis	GZCC 22-2009^{T}	OP508734	OP508774	OP698085	OP698074
Neohelicomyces hainanensis	GZCC 22-2027	OP508735	OP508775	OP698086	OP698075
Neohelicomyces helicosporus	GZCC 23-0633^{T}	PP512971	PP512975	PP526729	PP526735
Neohelicomyces helicosporus	GZCC 23-0634	PP512972	PP512976	PP526730	PP526736
Neohelicomyces hyalosporus	GZCC 16-0086^{T}	MH558745	MH558870	MH550936	MH551064
Neohelicomyces hydei	GZCC 23-0727^{T}	NA	PP512977	PP526731	PP526737
Neohelicomyces hydei	GZCC 23-0728	NA	PP512978	PP526732	PP526738
Neohelicomyces lignicola	CGMCC 3.25551^{T}	PP626600	PP639456	PP596357	PP596483
Neohelicomyces longisetosus	NCYU 106H1-1-1^{T}	MT939303	NA	NA	NA
Neohelicomyces macrosporus	CGMCC 3.25552^{T}	PP626601	PP639457	PP596358	PP596484
Neohelicomyces maolanensis	GZCC 23-0079^{T}	NA	PQ098529	PQ490683	PQ490677
Neohelicomyces maolanensis	GZCC 24-0148	NA	PQ522500	PQ490682	PQ490676
Neohelicomyces melaleucæ	CPC 38042^{T}	MN562154	MN567661	MN556835	NA
Neohelicomyces melaleucæ	KUNCC 23-14314	PP664108	PP664112	NA	NA
Neohelicomyces pallidus	CBS 245.49	MH856510	NA	NA	NA
Neohelicomyces pallidus	CBS 271.52	AY916461	AY856887	NA	NA
Neohelicomyces pallidus	CBS 962.69	AY916460	AY856886	NA	NA
Neohelicomyces pandanicola	KUMCC 16-0143^{T}	MH275073	MH260307	MH412779	NA
Neohelicomyces puerensis	**GMBCC 2217^{T}**	**PQ737369**	**PX308846**	**PX314508**	**PX314512**
Neohelicomyces puerensis	**GMBCC 2218**	**PQ737370**	**PX308847**	**PX314509**	**PX314513**
Neohelicomyces qixingyænsis	CGMCC 3.25569^{T}	PP626602	PP639458	PP596359	PP596485
Neohelicomyces sexualis	HGUP 24-0021	PQ570844	PQ570861	NA	NA
Neohelicomyces subtropicus	GZCC 23-0076^{T}	PQ098492	PQ098530	PQ490685	PQ490679
Neohelicomyces subtropicus	GZCC 24-0147	PQ522498	PQ522501	PQ490684	PQ490678
Neohelicomyces thailandicus	MFLUCC 11-0005^{T}	NR_171882	MN913696	NA	NA

Fungal species	Strain numbers	ITS	LSU	*tef*1-α	*rpb*2
Neohelicomyces thailandicus	GZCC 23-0400	PP626603	PP639459	PP596360	PP596486
Neohelicomyces submersus	MFLUCC 16-1106^{T}	KY320530	KY320547	NA	MH551068
Neohelicomyces wuzhishanensis	GZCC 23-0410^{T}	PQ098494	PQ098532	PV768325	PV768334
Neohelicomyces wuzhishanensis	GZCC 24-0164	PV730409	PV730413	PV768326	PV768335
Neohelicomyces xiayadongensis	CGMCC 3.25568^{T}	PP626604	PP639460	PP596361	PP596487
Neohelicomyces xiayadongensis	MUCL 15702	AY916459	AY856873	NA	NA
Neohelicomyces yunnanensis	GZCC 23-0735^{T}	PP664109	PP664113	NA	NA
Neohelicosporium bambusicola	MFLUCC 21-0156^{T}	OL606157	OL606146	OL964517	OL964523
Neohelicosporium ellipsoideum	MFLUCC 16-0229^{T}	MH558748	MH558873	MH550939	MH551071
Neohelicosporium fusisporum	MFUCC 16-0642^{T}	MG017612	MG017613	MG017614	NA
Neohelicosporium guineensis	ZHKUCC 24-0113^{T}	PP860090	PP860102	PP858062	PP858074
Neohelicosporium hyalosporum	GZCC 16-0076^{T}	MF467923	MF467936	MF535249	MF535279
Neohelicosporium irregulare	MFLUCC 17-1796^{T}	MH558752	MH558877	MH550943	MH551075
Neohelicosporium krabiense	MFLUCC 16-0224^{T}	MH558754	MH558879	MH550945	MH551077
Neohelicosporium laxisporum	MFLUCC 17-2027^{T}	MH558755	MH558880	MH550946	MH551078
Tubeufia guttulata	GZCC 23-0404^{T}	OR030841	OR030834	OR046678	OR046684
Tubeufia guttulata	GZCC 23-0590	OR066413	OR066420	OR058859	OR058852
Tubeufia hainanensis	GZCC 22-2015^{T}	OR030842	OR030835	OR046679	OR046685
Tubeufia javanica	MFLUCC 12-0545^{T}	KJ880034	KJ880036	KJ880037	NA
Tubeufia krabiensis	MFLUCC 16-0228^{T}	MH558792	MH558917	MH550985	MH551118
Tubeufia latispora	MFLUCC 16-0027^{T}	KY092417	KY092412	KY117033	MH551119
Tubeufia laxispora	MFLUCC 16-0232^{T}	KY092413	KY092408	KY117029	MF535287
Tubeufia machærinæ	MFLUCC 17-0055	MH558795	MH558920	MH550988	MH551122
Tubeufia mackenziei	MFLUCC 16-0222^{T}	KY092415	KY092410	KY117031	MF535288
Tubeufia muriformis	GZCC 22-2039^{T}	OR030843	OR030836	OR046680	OR046686
Tubeufia nigroseptum	CGMCC 3.20430^{T}	MZ092716	MZ853187	OM022002	OM022001
Tubeufia pandanicola	MFLUCC 16-0321^{T}	MH275091	MH260325	NA	NA

Ronquist et al. 2012), under the Markov Chain Monte Carlo (MCMC) framework, with six chains running for 2,000,000 generations and trees sampled every 100th generation. The multi-gene phylogenetic trees were visualized using FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and refined in Adobe Illustrator CS6 Adobe Systems Inc., USA).

Results

Phylogenetic analysis results

The phylogenetic positions of our newly introduced species were determined based on multi-gene (ITS+LSU+*rpb*2+*tef*1-α) phylogenetic analysis. The concatenated sequence matrix comprised 3,126 characters (ITS: 1–597, LSU: 598–1,386, *rpb*2: 1,387–2,273, and *tef*1-α: 2,274–3,126) across 75 ingroup and two outgroup taxa, *Bezerromyces brasiliensis* (CBS 141545 and URM7411). Both the ML and BI analyses of the concatenated ITS, LSU, *tef*1-α, and *rpb*2 datasets yielded similar tree topologies. The best-scoring RAxML tree, with a final ML optimization likelihood value of -22094.627605, is presented. The matrix contained 1064 distinct alignment patterns, with 21.45% of the characters being undetermined or gaps. Estimated base frequencies were as follows: A = 0.247173, C = 0.247452, G = 0.260954, T = 0.244421; substitution rates: AC = 0.918770, AG = 4.818137, AT = 2.150864, CG = 0.889941,

CT = 8.655879, GT = 1.000000; gamma distribution shape parameter α = 0.176011. The phylogenetic tree resulting from RAxML analysis is shown in Fig. 1. With reference to the multi-gene phylogram (Fig. 1), our collections represent two distinct *Neohelicomyces* species within Tubeufiaceae. Our isolates, *N. coffeæ* (GMBCC 2225 and GMBCC 2226), cluster together with *N. edgeworthiæ* (CGMCC 3.25565) as a sister lineage receiving 100% ML/1.00 PP support. Two additional strains from our collections, *N. puerensis* (GMBCC 2217 and GMBCC 2218), belong to a sister lineage to *N. dehongensis* (MFLUCC 18-1029), with 89% ML/0.99 PP support. Comparisons of nucleotide sequences showed that *N. coffeæ* and *N. edgeworthiæ* differed by 14/550 bp (2.54%, 5 gaps) in ITS, 1/913 bp (0.1%, no gap) in LSU, 7/924 bp (0.86%, 1 gap) in *tef*1-α, and 15/1,009 bp (1.48%, 13 gaps) in *rpb*2. Likewise, *N. puerensis* and *N. dehongensis* differed by 34/933 bp (3.6%, no gaps) in ITS, 7/870 bp (0.8%, no gaps) in LSU, and 16/887 bp (1.8%, no gaps) in *tef*1-α.

The phylogenetic tree (Fig. 1) clearly resolves *Neohelicomyces* as a strongly supported monophyletic clade within Tubeufiaceae (98% ML/1.00 PP). All species of *Neohelicomyces* are well separated with statistical support, and several well-defined subclades are recovered, such as the grouping of *N. edgeworthiæ* with *N. aquisubtropicus* and *N. denticulatus* with *N. pandanicola*. The related genera *Muriuplucha*, *Tubeufia*, and *Neohelicosporium* are also recovered as distinct, well-supported lineages, clearly separated from *Neohelicomyces*.

Taxonomy

Neohelicomyces coffeae **M.Y. Han & S. Tibpromma, sp. nov.**

Index Fungorum: IF904424

Fig. 2

Etymology. The species epithet "*coffeæ*" refers to the host genus *Coffea*.

Holotype. GMB-W1490.

Description. ***Saprobic*** on dead branches of *C. arabica*. ***Sexual morph*:** Undetermined. ***Asexual morph*:** Hyphomycetous, helicosporous. ***Colonies*** on natural substrate superficial, effuse, gregarious, white, can be seen as a white bush with the naked eye. ***Mycelium*** partly immersed to superficial, composed of hyaline to pale brown, branched, septate, smooth hyphae. ***Colonies*** composed of numerous coiled, spirally twisted, hyaline conidia aggregated in fascicles, which reflect light and impart a glistening, sparkling appearance to the colony surface. ***Conidiophores*** 144.5–274 × 3.4–6.2 µm ($\bar{x}$ = 201 × 4.6 µm, *n* = 30), macronematous, mononematous, erect or sub-erect, produced singly or in groups, some arising from creeping hyphae, cylindrical, straight or slightly flexuous, occasionally branched, septate, subhyaline to pale brown, thick-walled. ***Conidiogenous cells*** 13.2–20.6 × 3.4–5.2 µm ($\bar{x}$ = 17 × 4.1 µm, *n* = 25), holoblastic, monoblastic or polyblastic, integrated, terminal or intercalary, cylindrical, with denticles, hyaline, smooth-walled, truncate at apex after conidial secession, subhyaline to pale brown, smooth-walled. ***Conidia*** solitary, acropleurogenous, helicoid, tapering towards the rounded ends, developing on a tooth-like protrusion, 13.5–19.4 µm diam. ($\bar{x}$ = 17.1 µm, *n* = 25) and conidial filament 2.1–3.3 µm wide ($\bar{x}$ = 2.8 µm, *n* = 25), 105.3–146.4 µm long

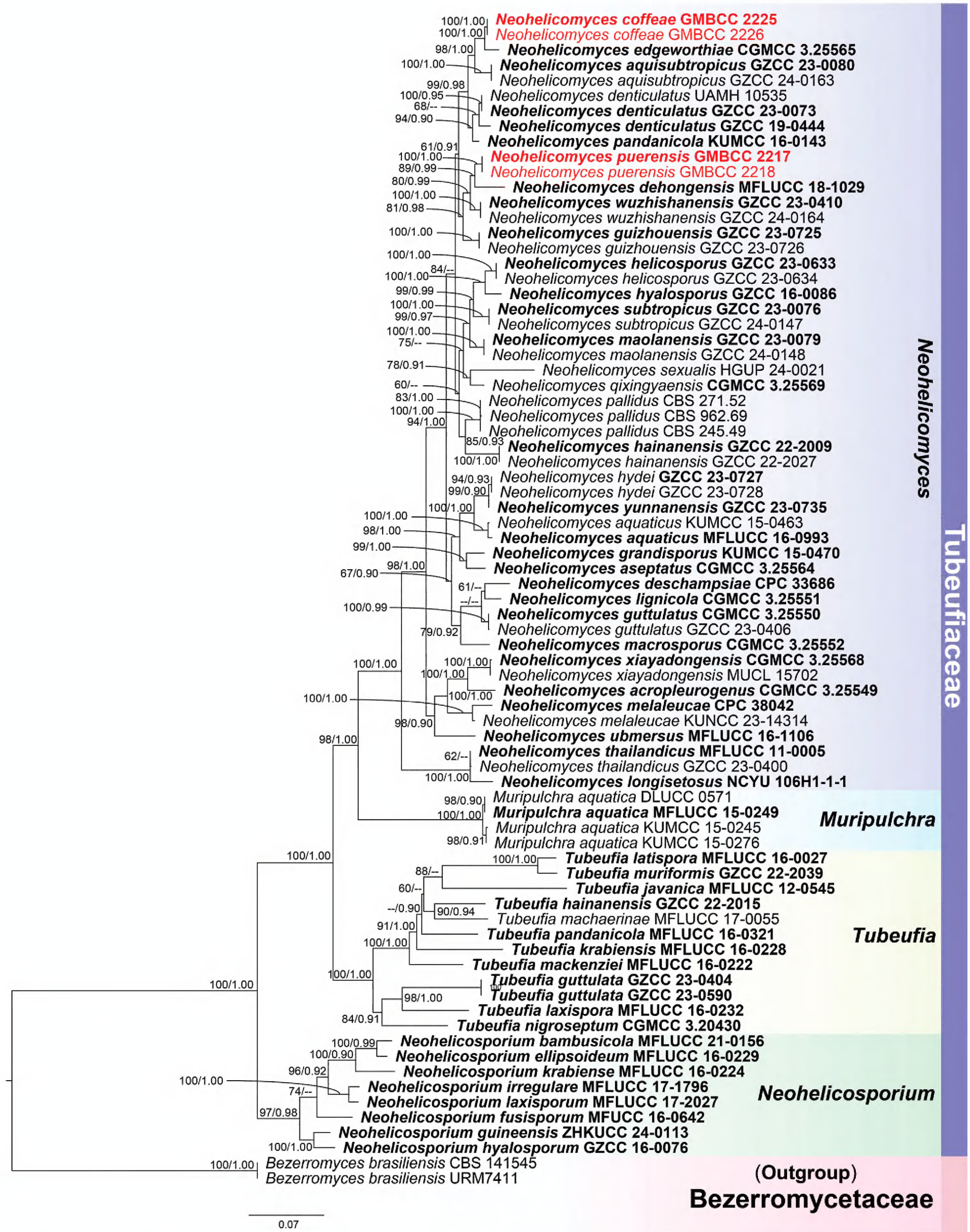

Figure 1. Phylogenetic tree generated from Maximum Likelihood (ML) analysis, based on the combined ITS+LSU+*rpb2*+*tef*1-α sequence data. Bootstrap support values of ML equal to or greater than 60% and Bayesian posterior probabilities (PP) equal to or greater than 0.90 are given near the nodes as ML/PP, respectively. The new species are typed in bold red, while the ex-type strains are in red.

Figure 2. *Neohelicomyces coffeæ* (GMB-W1490, holotype). **a, b.** Colonies on the host surface; **c.** Conidiophores, conidiogenous cells and conidia; **d.** Conidiogenous cells and conidia; **e, f.** Conidiogenous cells; **g–l.** Conidia; **m.** Germinated conidium; **n, o.** Surface and reverse colonies on PDA; **n** is the surface side, **o** is the reverse side. Scale bars: 50 μm (**c**); 25 mm (**n, o**); 20 μm (**d, m**); 10 μm (**e–l**).

($\bar{x}$ = 132 µm, *n* = 25), tightly coiled 2.5–3.75 times, loosely coiled three times, becoming loosely coiled in water, multi-septate, hyaline to subhyaline, some in pale brown, smooth-walled, granules.

Culture characteristics. Conidia germinated on PDA and produced germ tubes within 12 h. Colonies on PDA reaching 22 mm diam. after two weeks of incubation at 28 °C with an irregular shape, rough surface, slightly raised, radially striated with a lobate edge. Colony surface centrally dark brown to chestnut-brown, becoming paler towards the periphery; aerial mycelium sparse to moderate, whitish at the margin. Reverse side uniformly dark brown.

Material examined. CHINA • Yunnan Province, Pu'er City, Simao District (22°36'36"N, 101°0'14"E, 1189 m), on dead branches of *C. arabica* L., 1 August 2024, M.Y. Han, (BG55 = GMB-W1490, holotype), ex-type GMBCC 2225, other living culture GMBCC 2226.

GenBank number. GMBCC 2225 = ITS: PX308843, LSU: PX308848, *tef*1-α:PX314510, *rpb*2: PX314514 and GMBCC 2226 = ITS: PX308844, LSU: PX308849, *tef*1-α: PX314511, *rpb*2: PX314515.

Notes. In the phylogenetic analyses (Fig. 1), our collections of *N. coffeæ* [GMBCC 2225 (ex-type) and GMBCC 2226] clustered with *N. edgeworthiæ* (CGMCC 3.25565) as a sister lineage, receiving 100% ML/1.00 PP support. Comparisons of nucleotide sequences between *N. coffeæ* and *N. edgeworthiæ* revealed minor overall differences; however, the ITS region showed a 14/550 bp (2.54%, 5 gaps) difference. Morphologically, the conidiogenous cells of our isolates are notably longer than those of *N. edgeworthiæ* (13.2–20.6 µm vs. 9–14 µm) (Ma et al. 2024b). Conversely, the conidia of our collections are significantly smaller than those of *N. edgeworthus*, both in diameter (13.5–19.4 µm vs. 21.5–34 µm) and length (105.3–146.4 µm vs. 121–177 µm) (Ma et al. 2024b). In addition, the conidia of *N. edgeworthiæ* are aseptate and have numerous guttulate, whereas conidia of our collections are multi-septate, lack guttulate, and have only surface granules (Ma et al. 2024b). In summary, although the nucleotide divergence between our collections and *N. edgeworthiæ* is relatively low, the combination of distinct morphological characters and phylogenetic position supports their separation, and therefore, we introduce *N. coffeæ* herein as a novel species.

Neohelicomyces puerensis **M.Y. Han & Tibpromma S., sp. nov.**

Index Fungorum: IF904425

Fig. 3

Etymology. The species name "*puerensis*" refers to the type locality, Pu'er City, China.

Holotype. GMB-W1485.

Description. ***Saprobic*** on dead branches of *C. arabica*. Sexual morph Undetermined. ***Asexual morph*** Hyphomycetous, helicosporous. ***Colonies*** on natural substrate superficial, effuse, gregarious, white, can be seen as a white bush with the naked eye. ***Mycelium*** partly immersed, partly superficial, composed of hyaline to pale brown, branched, septate, smooth hyphae, comprising glistening conidial mass. ***Conidiophores*** 138.8–211.7 × 2.6–4.2 µm ($\bar{x}$ = 182.4 × 3.4 µm, *n* = 30), macronematous, mononematous, some arising from creeping hyphae, erect, cylindrical, straight or slightly flexuous, unbranched or occasion-

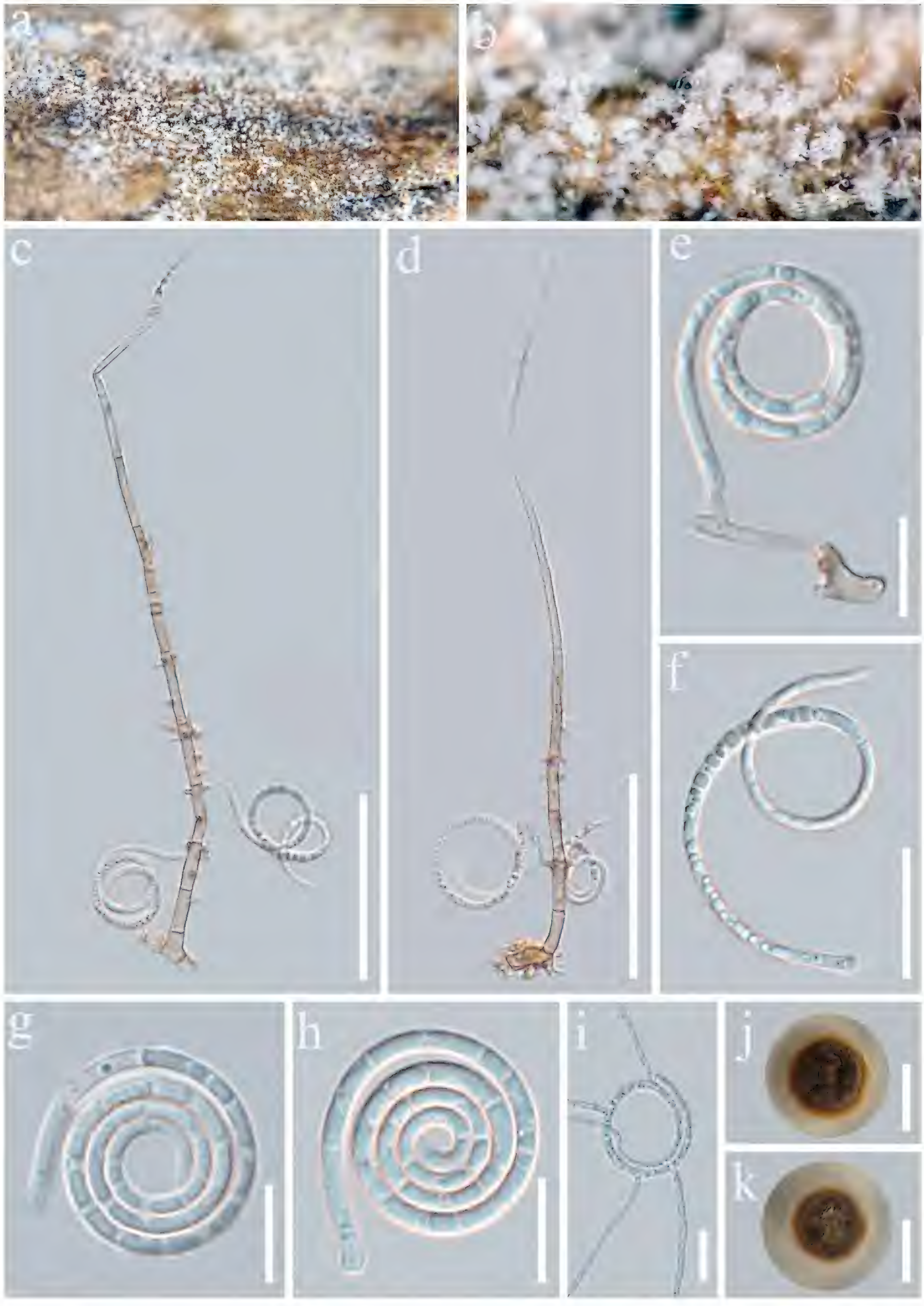

Figure 3. *Neohelicomyces puerensis* (GMB-W1485, holotype). **a, b.** Colonies on the host surface; **c, d.** Conidiophores, conidiogenous cells, and conidia; **e.** Conidiogenous cells and conidia; **f–h.** Conidia; **i.** Cerminated conidium; **j, k.** Surface and reverse colonies on PDA. Scale bars: 50 μm (**c, d**); 25 mm (**j, k**), 20 μm (**f, i**), 10 μm (**e, g, h**).

ally branched, septate, pale brown to brown at base, paler towards the apex, thick-walled. ***Conidiogenous cells*** 11.6–20 × 2.4–4.1 µm ($\bar{x}$ = 14.8 × 3.2 µm, *n* = 25), holoblastic, monoblastic or polyblastic, integrated, terminal or intercalary, cylindrical, with denticles, hyaline to pale brown, smooth-walled. ***Conidia*** solitary, acropleurogenous, helicoid, tapering towards the rounded ends, developing on a tooth-like protrusion, 15.2–22 µm diam. ($\bar{x}$ = 17.94 µm, *n* = 25) and conidial filament 1.9–3.7 µm wide ($\bar{x}$ = 2.5 µm, *n* = 20), 106.6–211.2 µm long ($\bar{x}$ = 138 µm, *n* = 30), tightly coiled 2.5–4.25 times, becoming loosely coiled in water, multi-septate, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia germinated on PDA and produced germ tubes within 10 h. Colonies on PDA reaching 32 mm diam. after 30 days of incubation at 25 °C. The colony is regularly circular in shape, with a convex surface; the central part is cracked, resembling a petal-like appearance, dark gray to brownish, with a pale brown margin. Reverse side is dark brown, with the margin varying from brown to white.

Material examined. CHINA • Yunnan Province, Pu'er City, Simao District (22°36'36"N, 101°0'14"E, 1189 m), on dead branches of *C. arabica*, 1 August 2024, M.Y. Han, (BG21 = GMB-W1485, holotype), ex-type GMBCC 2217, other living culture GMBCC 2218.

GenBank number. GMBCC 2217 = ITS: PQ737369, LSU: PX308846, *tef*1-α: PX314508, *rpb*2: PX314512 and GMBCC 2218 = ITS: PQ737370, LSU: PX308847, *tef*1-α: PX314509, *rpb*2: PX314513.

Notes. In the concatenated phylogenetic analysis, our strains [GMBCC 2217 (ex-type) and GMBCC 2218] of *N. puerensis* formed a sister branch to *N. dehongensis* (MFLUCC 18-1029) with 89% ML/0.99 PP statistical support (Fig. 1). Sequence comparisons between *N. coffeæ* and *N. dehongensis* revealed greater divergence in the ITS region (34/933 bp; 3.6%, no gap). Morphologically, our species resembles *N. dehongensis* in having macronematous and mononematous conidiophores, holoblastic conidiogenous cells with denticles, and helicoid, multi-septate, hyaline conidia (Dong et al. 2020). However, it differs from *N. dehongensis* in having slightly shorter conidiophores (138.8–211.7 µm vs. 120–250 µm) and conidia (106.6–211.2 µm vs. 145–210 µm), as well as narrower conidial diameters (15.2–22 µm vs. 20–25 µm) (Dong et al. 2020). In addition, conidia of *N. puerensis* are distinctly multi-septate, whereas those of *N. dehongensis* are indistinctly septate. The conidial filaments in our collections are more tightly coiled (2.5–4.25 times vs. 2.75–3.75 times), and conidiophores are usually unbranched, lacking the basal forking that is frequently observed in *N. dehongensis*. Moreover, conidiogenous cells of *N. puerensis* are terminal or intercalary, whereas those of *N. dehongensis* are mostly intercalary and sympodial. Thus, based on these morphological differences and molecular evidence, we propose that *N. puerensis* be recognized as a new species.

Discussion

Coffee ecosystems host diverse fungal assemblages, including pathogens, endophytes, and saprophytes (McCook and Vandermeer 2015; Lu et al. 2022a, 2025a, 2025b). Although saprophytic fungi account for a relatively small proportion of these communities (Lu et al. 2022a, 2025a, 2025b), they

play critical roles in litter decomposition, nutrient cycling, and maintaining ecosystem stability (Liu et al. 2022; Zhang et al. 2023a). Recent surveys of coffee agroecosystems have revealed a rapid increase in previously unreported saprophytic fungi, with the known record expanding to more than 150 species, including numerous new taxa and new records (Lu et al. 2022a, 2025a, 2025b; Han et al. 2024, 2025a, 2025b).

Beyond their ecological functions, many saprophytic fungi are also recognized as promising sources of bioactive metabolites with potential applications in medicine and agriculture (Bills and Gloer 2016; Lu et al. 2018b; Keller 2019; Zhang et al. 2024). In particular, members of the family Tubeufiaceae have been reported to produce a range of bioactive secondary metabolites with antifungal, antibacterial, antidiabetic, and anticancer activities (Itazaki et al. 1990; Hanada et al. 1996; Hu et al. 2006; Lu et al. 2018b; Zhang et al. 2023b, 2024). Within this family, taxonomic studies of *Neohelicomyces* have not only clarified species diversity and distribution, but also highlighted their chemical potential (Zheng et al. 2023; Ma et al. 2024a, 2024b). For example, compounds isolated from *N. hyalosporus* (PF11-1) exhibited moderate cytotoxicity against human cancer cell lines, including A549, TCA, and RD (Zheng et al. 2023). These findings suggest that *Neohelicomyces* could provide valuable candidate strains for the development of oncology-related drugs.

According to Index Fungorum (2026), *Neohelicomyces* currently comprises 36 species distributed across Asia (especially China (35/36 = 97.22%), with Guizhou Province as a diversity hotspot, following by Yunnan, Guangxi, Hainan, Tibet, Guangdong, and other regions), Europe (2/36 = 5.56%), and North America (2/36 = 5.56%) (Note: percentages are calculated as the proportion of the 36 accepted species that have been reported from each region; species occurring in multiple regions were counted in each relevant region) (Luo et al. 2017; Tibpromma et al. 2018; Crous et al. 2019a, 2019b; Dong et al. 2020; Hsieh et al. 2021; Lu et al. 2022b; Yang et al. 2023; Ma et al. 2024a, 2024b, Ma et al. 2025; Peng et al. 2025; Sun et al. 2025). Most species inhabit freshwater driftwood or moist substrates, e.g., *N. aquaticus*, *N. grandisporus*, *N. submersus* (Luo et al. 2017), *N. thailandicus* (Dong et al. 2020), *N. hydei*, *N. lignicola*, *N. macrosporus* (Ma et al. 2024a, 2024b), while others occur on terrestrial plant tissues, e.g., *N. melaleucæ* on leaves (Crous et al. 2019b), *N. pandanicola* on roots (Tibpromma et al. 2018). In particular, *N. pallidus* has been recorded from multiple countries, including China, the Czech Republic, Italy, Japan, the Netherlands, and the United States, and exhibits both freshwater and terrestrial occurrences (Linder 1929; Goos 1989; Tsui et al. 2001; Zhao et al. 2007; Lu et al. 2018b; Ma et al. 2024b). This ecological versatility suggests strong adaptive potential.

In this study, we report two novel *Neohelicomyces* species (*N. coffeæ* and *N. puerensis*) isolated from decaying branches of *C. arabica*. This discovery marks the first records of new taxa of this genus from coffee hosts, thereby extending its known host and substrate range into agricultural ecosystems. Morphologically, species of *Neohelicomyces* are generally similar, but they can be differentiated by several diagnostic features, such as the size of conidiophores and conidia, the diameter and times of coils of conidia, the presence or absence of septa in conidia, and whether conidiophores are branched. In addition to these morphological characteristics, DNA sequence data, partic-

ularly from the ITS region, provide robust evidence for species delimitation. Beyond their taxonomic significance, these species may represent candidate strains for the exploration of bioactive secondary metabolites. Furthermore, by expanding the known diversity of *Neohelicomyces* in agricultural environments, our findings provide a foundation for future studies that integrate taxonomy, ecology, and natural product research, and highlight the potential of saprophytic fungi as reservoirs of novel metabolites with pharmaceutical and biotechnological applications (Strobel and Daisy 2003; Duong et al. 2020; Zheng et al. 2023; Han et al. 2025a).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

Conceptualization: ST, NK. Data curation: ST, MYH, LL. Formal analysis: SCK, ST, and NS. Funding acquisition: SCK, ST, JYY, and AME. Investigation: SCK and MYH. Methodology: MYH, ST, NS. Project administration: ST, SCK, and NS. Resources: MYH. Software: MYH, LL, DGZ, and LJZ. Supervision: ST NS, and SCK. Validation: ST, NS, and SCK. Visualization: ST and MYH. Writing - original draft: MYH. Review and editing: ST, JYY, SCK, NS, JK, LL, DGZ, AME, FQY, ATA, DQD, LJZ.

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Data availability

All of the data that support the findings of this study are available in the main text.

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